

ENVIRONMENTAL REGULATION OF THE ANNUAL  
REPRODUCTIVE SEASON OF *STRONGYLOCENTROTUS*  
*PURPURATUS* (STIMPSON)

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Along the length of the Pacific Coast from Alaska to Cedros Island, the gonads of *Strongylocentrotus purpuratus* contain numerous mature gametes during the winter months (Lasker and Giese, 1954; Bennett and Giese, 1955; Holland and Giese, 1965; Boolootian, 1966; Chatlynne, 1969; Cochran and Engelmann, 1972; Gonor, 1973a, 1973b). Following this season, the gonads are depleted of gametes, and nutritional reserves appear to be accumulated (Holland and Giese, 1965; Gonor, 1973a). In the fall and early winter months, more and more gametes are produced and accumulate. The synchrony of the annual reproductive cycles of different populations of *S. purpuratus* has led to suggestions that they are regulated by environmental factors (Bennett and Giese, 1955; Boolootian, 1963; Chatlynne, 1969; Gonor, 1973a; Giese and Pearse, 1974).

Boolootian (1963) reported a proliferation of spermatogonia under the stimulus of long-day photoperiod (14L:10D), while short-day (6L:18D) presumably induced complete maturation of sperm within three weeks. Others (Bennett and Giese, 1955; Chatlynne, 1969) have suggested that temperature, or food availability (Gonor, 1973a) may be the agents regulating the reproductive cycle. On the other hand, it has been reported that a laboratory population of *S. purpuratus* in constant temperature ( $15^{\circ} \pm 1^{\circ}$  C) and light, approximating natural conditions, remained reproductively synchronous with the field populations over a three year period (Boolootian, 1964). This latter information raises the possibility of an endogenous near annual rhythm of reproduction, independent of environmental factors (Boolootian, 1966).

In conjunction with research on echinoid spawning factors (Cochran and Engelmann, 1972), the current study was undertaken to analyze the effects of photoperiod and/or temperature on the onset and termination of the reproductive activity of a Southern California subtidal population of *S. purpuratus*. Subtidal animals were chosen because changes in environmental conditions, such as temperature, salinity, and food availability, are generally more gradual than in the intertidal zones.

MATERIALS AND METHODS

All specimens of *S. purpuratus* were collected at Flat Rock Point on the Palos Verdes Peninsula (Los Angeles, California) at depths of two to eight meters using SCUBA and skin-diving equipment. The animals were transported in burlap bags to holding tanks within one hour of collection. Approximately  $\frac{1}{3}$  of the animals collected in this manner die within the first two weeks, but there is not much subsequent attrition.

The following arrangement was used for the photoperiod experiments: four 150-liter tanks were arranged in two tiers of two tanks each. All external light was excluded from the tanks by means of heavy, opaque, rubberized curtains. The top tier was insulated from the light of the bottom tier as well. Light was provided by two 100-watt tungsten-filament bulbs over each tank, and controlled by timer clocks. The light cycle for the top tier was independent from the cycle of the bottom tier. The artificial light:dark cycles used here corresponded to the photoperiods of the summer and winter solstices at Flat Rock Point, 14L:10D and 10L:14D respectively. Sea water in the tanks was obtained from a general 40,000 liter sea water system, which continuously cooled, filtered, and aerated the water. Additional aeration was provided by two airstones in each tank. Fresh, brown algae (*Egrecia* sp.) was given weekly as food for the sea urchins.

Animals were considered reproductively active if mature gametes, discernible with the unaided eye, oozed from the severed gonads (Cochran and Engelmann, 1972). Reproductively active and inactive sea urchins were exposed to either long- or short-day photoperiods for up to 90 days. Animals used in each experiment were collected not earlier than two days prior to the initiation of an experiment. At the end of all experiments, sea urchins were cut open to ascertain their reproductive condition. Animals taken from the ocean at the time these experiments were terminated were used for comparison.

For the temperature experiments, the water temperature in one tank was modified by a Lauda thermostat refrigeration and pump unit. Low temperature (13° C) was maintained for up to 90 days, and high temperature (19° C) for 30 days. These temperatures approximate the minimum and maximum ocean temperatures encountered by the subtidal sea urchin population at Flat Rock Point. The temperature of the water of the second tank in the same tier was that of the general sea water system (average 14° C). Animals were sampled from time to time to ascertain their reproductive condition.

## RESULTS

### *Photoperiod*

In the first experiment, begun September 19, 1970, 30 reproductively inactive animals were placed in each of the four tanks. The photoperiod of the top tier was 10L:14D, and that of the bottom tier was 14L:10D. The experiment was terminated six weeks later on November 1, 1970. A subsequent experiment was begun November 8, 1970 with 50 animals (27% reproductively active) per tank under the same photoperiod regimes. This experiment was terminated 13 weeks later on February 14, 1971. The water temperature during these and following photoperiod experiments averaged 13° C, with a range from 12 to 14.5° C. As is seen (Table I), neither long- (14L:10D) nor short-day (10L:14D) photoperiods affected the onset of reproductive activity, either accelerating or inhibiting the appearance of spawnable gametes. In none of the experimental series was the reproductive activity significantly different from that of the subtidal field populations. A marginally significant difference was, however, found between the long- and short-day photoperiod populations in the experiments ending November 1,

TABLE I

*The effect of long- and short-day photoperiod on onset or termination of spawning capability. Comparison between the two laboratory populations (P), and between laboratory and field populations (P') (Chi square).*

Photoperiod	Duration of experiment (weeks)	Laboratory animals		Ocean animals	
		Active/total	P	Active/total	P'
10L:14D	6	17/46	<2.5%	8/30	<40%
14L:10D	6	5/35			<30%
10L:14D	13	73/76	<20%	20/20	<50%
14L:10D	13	57/63			<20%

1970. No difference was found between the long- and short-day photoperiod populations of the second experiment ending February 14, 1971.

A second series of experiments was begun on July 14, 1971, when the animals were in their early reproductively inactive phase (Cochran and Engelmann, 1972). Fifty sea urchins were placed in each of the four tanks, and the photoperiods of the two tiers were left the same as in the previous experiments. Animals from the top tier (10L:14D) were sacrificed 10 weeks later (October 1, 1971). The proportion of reproductively active animals in these short-day populations was not significantly different from that of the field population (Table II). Obviously the 10 weeks of short-day treatment did not induce early reproductive activity. New animals from the field were placed in the top tier (50 per tank) at this time, and the photoperiod was changed to 14L:10D, the same as the bottom tier. All sea urchins from the four tanks were then sacrificed on December 12, 1971. The reproductive activity of animals kept for only 10 weeks under long-day photoperiod was not significantly different from that of animals kept for 20 weeks under

TABLE II

*Effects of extended long- and short-day photoperiods on the reproductive activity of the purple sea urchin. P values (chi square) are obtained for a comparison between the laboratory and ocean populations.*

Photoperiod	Duration of experiment (weeks)	Laboratory animals Active, total	Ocean animals at termination Active total	P
10L:14D	10	3/21	0/20	<10%
10L:14D	10	4/28		<10%
14L:10D	10	32/36	20/20	<20%
14L:10D	10	34/38		<20%
14L:10D	20	20/21		<50%
14L:10D	20	21/23		<20%

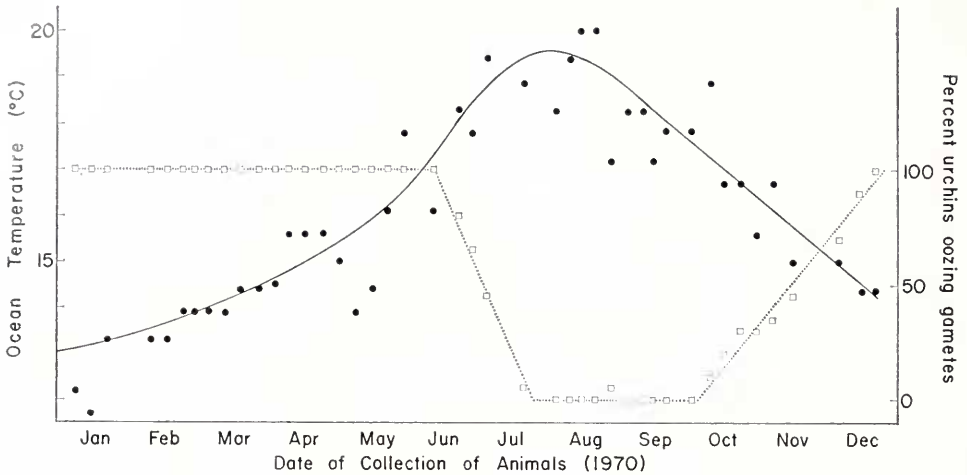


FIGURE 1. Annual reproductive cycle of *S. purpuratus*, as measured by the percentage of animals that released gametes when cut open (dotted curve) superimposed on the seasonal variation in ocean temperature (solid line) at the same location. Subtidal ocean temperatures were taken on the dates of collection.

similar conditions. The reproductive activity of the two laboratory populations did not differ from that of the field population. Thus long-day photoperiod (14L:10D) did not inhibit the onset of reproductive activity. It should be mentioned here that the water temperature ( $13^{\circ}\text{C}$ ) in the holding tanks was markedly lower than that in the field throughout most of this period (Figure 1).

### Temperature

It became apparent during these studies that the annual reproductive cycle of *S. purpuratus* from Palos Verdes is inversely correlated with the annual fluctuation of ocean temperature at the same location (Figure 1). A rise of water temperature above  $17^{\circ}\text{C}$  coincides with the loss of the ability to spawn. A series of experiments were thus undertaken in order to ascertain whether temperature is indeed the environmental cue for the regulation of the reproductive season of this population.

In an experiment begun May 1, 1972, almost all animals maintained at  $13^{\circ}\text{C}$  (the normal winter temperature) and a photoperiod of 14L:10D remained reproductively active for nearly two months beyond the date (late June) when animals in the field had become reproductively inactive (Table III). During this experiment, for a period of 10 days in late July, the temperature of the general sea water system accidentally rose to  $17.5^{\circ}\text{C}$ , while that of the regulated tank reached  $16.5^{\circ}\text{C}$ . Prior to, and after this period, the temperature of the general sea water system averaged  $14.5^{\circ}\text{C}$  ( $13.5^{\circ}$  to  $15^{\circ}\text{C}$ ) and that of the regulated tank  $13^{\circ}\text{C}$  ( $12.5^{\circ}$  to  $13.5^{\circ}\text{C}$ ). Before this short term rise in temperature, there had been no significant difference between the proportions of reproductively active animals in the two laboratory populations. The proportion of reproductively active animals in

TABLE III

*Effects of low temperature on reproductive activity in S. purpuratus.*

	Elapsed time (weeks)	Experimental tanks			Sea water system			Ocean		
		Temp. (° C)	Sea urchins Active/total	%	Temp. (° C)	Sea urchins Active/total	%	Temp. (° C)	Sea urchins Active/total	%
At initiation	0	13			15			13.9	30/30	100
First sampling	7	13	9/10	90	14	10/10	100	17.8	2/20	10
Second sampling	10	13.5	9/10	90	14	8/10	80	18.9	2/20	10
Third sampling	12	16.5*	10/10	100	17.5*	5/10	50	20.5	0/20	0
Fourth sampling	14	12.5	13/14	93	14	6/17	33	20.5	0/20	0

\* For a period of 10 days in late July the temperature of the general sea water system reached 17.5° C, while that of the experimental tanks was 16.5° C.

both of these laboratory populations was significantly different from that of the ocean population ( $P < 0.5\%$ ). The rise in temperature to 17.5° C caused a significant ( $P < 1\%$ ) reduction in the proportion of reproductively active animals compared to the other laboratory animals at 16.5° C. This difference in reproductive activity between the two laboratory populations was even more significant ( $P < 0.5\%$ ) at the time the experiment was terminated. These results indeed suggest that reproductive activity in this species of sea urchin is turned off by an elevated temperature.

In a further effort to test the hypothesis that warm temperature terminates reproductive activity, 50 animals were placed in each of two adjacent tanks (water temperature 12.5° C) during the height of the reproductive season (February 11, 1973). In the course of 11 days the water temperature in one tank was raised to 19° C. Three weeks later, nearly all surviving animals had either spawned or resorbed their gametes (Table IV). During this entire period the animals were kept in short-day photoperiod (10L:14D).

The converse experiment, *i.e.*, induction of reproductive activity by cool water temperature, was started on September 15, 1972 with 50 animals in each tank. After eight weeks of low temperature (13° C), the proportion of reproductively active animals in either tank was not significantly different from that of the field population (Table V). Also, an experiment started in July of 1970 to test the effects of short-day photoperiod was performed at 13° C for 10 weeks (Table II).

TABLE IV

*Reproductive activity of S. purpuratus as affected by elevated temperature.*

	Experimental tanks			Sea water system			Ocean		
	Temp. (° C)	Sea urchins Active/total	%	Temp. (° C)	Sea urchins Active/total	%	Temp. (° C)	Sea urchins Active/total	%
At initiation	17			12.5			13.3	20/20	100
At termination	19	1/20	5	12.5	42/43	98	13.3	40/40	100

TABLE V

*Effect of reduced temperature on inducing reproductive activity in S. purpuratus during the late inactive season.*

	Elapsed time (weeks)	Experimental tanks			Sea water system			Ocean		
		Temp. (° C)	Sea urchins Active/total	%	Temp. (° C)	Sea urchins Active/total	%	Temp. (° C)	Sea urchins Active/total	%
At initiation	0	13			14			20.5	0/20	0
First sampling	7	12.5	0/20	0	14	0/20	0	16.1	3/20	15
Second sampling	8	13.5	2/20	10	15	3/20	15	15.1	5/20	25

Here too, the proportion of reproductively active animals was not significantly different from that of the field population. Thus, under these experimental laboratory conditions, the onset of spawning capability was not hastened.

Observations made in the field study area during 1973 tend to support the concept of temperature-regulated reproductive activity in *S. purpuratus*. Animals collected during March and April of 1973 were reproductively inactive (an unusual phenomenon for this season) and the gonads had the spent appearance normally observed at the end of the reproductive season. However, subsequent to May 11, all of the sea urchins collected had copious amounts of gametes. This renewal of reproductive activity, following a "spawn out" (possibly caused by low-salinity shock or turbulence from an unusually high number of rain storms passing through the area) occurred while the field population of sea urchins was still experiencing low temperatures. Even through the summer months of 1973, 10% of the sea urchin population retained spawning capability, which correlated with an unusually cold summer ocean temperature.

## DISCUSSION

The designation, reproductive season, breeding season, or reproductive period is generally based on the gonad index, presence of numerous mature gametes, or size- and stage-frequency analysis of oocytes in *S. purpuratus* (Booolootian, 1966). Each of these methods, however useful, has limitations (Gonor, 1972, 1973a; Giese and Pearse, 1974). The first appearance of numerous mature gametes, coupled with a high gonad index, signals the onset of the reproductive season. The actual frequency of spawning by *S. purpuratus* field populations during this period is unknown. Studies on gametogenesis reveal a continuous maturation of gametes during the reproductive season which makes possible several spawning events (Holland and Giese, 1965; Chatlynne, 1969). The spawned out appearance, which marks the end of the reproductive season, is caused by released or resorbed gametes not being completely replaced by new ones (Chatlynne, 1969; Gonor, 1973a, 1973b).

As many observations suggest (Bennett and Giese, 1955; Booolootian, 1966; Gonor, 1973a), the reproductive season of *S. purpuratus* is probably controlled by environmental factors. Figure 1 illustrates the annual reproductive cycle for

1970 in the Palos Verdes subtidal population. A drop in spawning capability occurs around the summer solstice, while full reproductive activity is achieved a couple of weeks before the winter solstice. This seemingly agrees with the hypothesis that reproductive activity of *S. purpuratus* is affected by changing photoperiods (Booolootian, 1963). However, the main body of data given in this paper (Tables I, II) indicates that short-day photoperiod (10L:14D) does not induce the onset of reproductive activity. Only 14% of the animals in populations kept under short-day photoperiod (10L:14D) for 10 weeks during the summer became reproductively active, and this was not significantly different from the reproductive activity of the ocean population (Table II). One might expect a much greater proportion of reproductively active animals since the maturation and growth of ova from primary oocytes can be completed in approximately this time (Pearse, Clark, Leighton, Mitchell, and North, 1970), and the maturation of sperm under short-day photoperiod (6L:18D) takes only three weeks (Booolootian, 1963). Conversely, the reproductive activity of animals kept under long-day photoperiod (14L:10D) from July 19 until December 12, 1971 (the beginning of the natural reproductive season) was not significantly different from the subtidal population. Thus the onset of reproductive activity was not retarded by the lack of short-day photoperiod. The marginally significant difference ( $P < 2.5\%$ ) in the proportion of reproductively active animals between two experimental populations kept under short- and long-day photoperiods respectively while at  $13.5^{\circ}\text{C}$  (Table I) cannot be overlooked. The preponderance of evidence, however, suggests that photoperiod has no direct influence on initiation or termination of reproductive activity.

The annual oscillation in ocean temperature has been suggested as a possible cue for synchronizing an endogenous reproductive rhythm in *S. purpuratus* (Bennett and Giese, 1955; Chatlynne, 1969). In Figure 1, ocean temperature and reproductive activity of *S. purpuratus* during 1970 are superimposed. In the laboratory, reproductive activity could be maintained by low temperature ( $13^{\circ}\text{C}$ ) (Table III), and terminated by warm temperature ( $19^{\circ}\text{C}$ ) (Table IV). These findings are in agreement with the results obtained by Dr. John Pearse on the same species (personal communication). He has demonstrated that sea urchins kept for one month at  $7^{\circ}\text{C}$  or  $14^{\circ}\text{C}$  retained copious gametes in the gonads, while those at  $21^{\circ}\text{C}$  resorbed most of their gametes. Thus, there is substantial evidence that the reproductive season of *S. purpuratus* is terminated by increased temperature.

The untimely loss of reproductive activity during March and April of 1973 (possibly caused by low-salinity shock or turbulence from an unusually high number of rain storms passing through the area) occurred while the ocean temperature was less than  $14.5^{\circ}\text{C}$ . Normally in the Palos Verdes area, a population "spawn out" does not occur until mid-June, accompanied by a rise in ocean temperature above  $17^{\circ}\text{C}$ . Perhaps this rise in temperature induces spawning (Giese and Pearse, 1974), and does not allow a continued gamete maturation. During late spring of 1973, however, the sea urchins regained full reproductive activity within a few weeks, an indication that the absence of gametes was due to frequent spawning and that gamete maturation had not abated. These field observations thus support the hypothesis that reproductive activity is halted by increased ocean temperature.

While the control of the termination of reproductive activity has been elucidated in at least one case, the environmental factor(s) regulating the onset of reproductive activity has yet to be determined. In the laboratory, neither short-day photoperiod (10L:14D) nor low temperature (13° C) accelerated the onset of reproductive activity in this sea urchin (Tables II, IV). This raises the possibility that the control of the onset of reproductive activity is more complex than we originally suspected. For example, the duration of the inactive period that follows the reproductive season may be a function of the time necessary to acquire sufficient energy reserves to generate mature gametes. In support of this hypothesis one can cite the northern intertidal populations of *S. purpuratus*, which depend upon seasonally varying amounts of algal drift (Gonor, 1973a). This population is reproductively inactive longer than southern, subtidal sea urchins, which continuously obtain their nutrition by grazing on algae-encrusted rocks (Pearse, Clark, Leighton, Mitchell, and North, 1970; Leighton, 1971).

Although ocean temperature plays a role in the reproductive activity of a Southern California subtidal population of *S. purpuratus*, it may not be the only cue for other populations. Northern, subtidal sea urchins, not exposed to the high temperatures of southern latitudes, may rely on a different critical temperature. Also, intertidal populations, both north and south, experience frequent fluctuations in temperature, salinity, and food availability and thus might be expected to cue on a more predictable environmental factor, such as photoperiod. However, at present no information is available to show that this is indeed the case.

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#### SUMMARY

1. The annual reproductive cycle of the Palos Verdes (Southern California) subtidal population of *S. purpuratus* correlates with seasonal changes in ocean temperature: a rise of temperature above 17° C (June) coincides with cessation of gamete production and storage.

2. Sea urchins maintained at low temperature (13° C) retained gametes nearly two months (as long as they were observed) beyond the date when the field population had "spawned out."

3. At a time when the field population was reproductively active, the spawning capability of a laboratory population was terminated by three weeks of warm water temperature (19° C).

4. The spawning capability of animals kept at 13° C was not terminated by long-day photoperiod (14L:10D).

5. The onset of reproductive activity in animals started during the summer months could not be accelerated experimentally by 10 weeks of low temperature (13° C).

6. In laboratory animals maintained at 13° C, the onset of reproductive activity was neither enhanced by short-day (10L:14D), nor retarded by long-day (14L:10D) photoperiod.



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